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ORIGINAL ARTICLE

Phylogenetic and ecological correlates of pollen morphological diversity in a Neotropical rainforest

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Abstract

Morphology varies enormously across clades, and the morphology of a trait may reflect ecological function or the retention of ancestral features. We examine the tension between ecological and phylogenetic correlates of morphological diversity through a case study of pollen grains produced by angiosperms in Barro Colorado Island, Panama (BCI). Using a molecular phylogeny of 730 taxa, we demonstrate a statistically significant association between morphological and genetic distance for these plants. However, the relationship is non-linear, and while close relatives share more morphological features than distant relatives, above a genetic distance of ~0.7 increasingly distant relatives are not more divergent in phenotype. The pollen grains of biotically pollinated and abiotically pollinated plants overlap in morphological space, but certain pollen morphotypes and individual morphological traits are unique to these pollination ecologies. Our data show that the pollen grains of biotically pollinated plants are significantly more morphologically diverse than those of abiotically pollinated plants.

Abstract in Spanish is available with online material.

KEYWORDS

disparity, morphospace, phylogeny, pollination, tropical rainforests

1 | INTRODUCTION

Morphological diversity varies strikingly across clades and morphological differences between taxa are thought to evolve for a variety of reasons (Vidal-García, Byrne, Roberts, & Keogh, 2014). For example, ecology and morphology can be linked by common functional demands (Miles & Ricklefs, 1984), and variation in morphology may bear a strong relationship to variation in ecological characteristics (Losos & Miles, 1994) to the point that morphology has been used to indicate ecological function (Ricklefs, 2012). However, the form

of a morphological trait also partly reflects evolutionary descent and the retention of ancestral features (Losos & Miles, 1994; Verde Arregoitia, Fisher, & Schweizer, 2017), and it has been shown that morphological dissimilarity and evolutionary divergence time can be positively correlated (Losos, 2008; Mouquet et al., 2012). Detailed studies of ecology, phylogeny, and morphological diversity of taxa can shed light on the relationship between phylogenetic relatedness and phenotypic similarity (Losos, 2008), and inform conservation strategies that aim to conserve evolutionary history and phenotypic variation (Kelly, Grenyer, & Scotland, 2014). Such knowledge is

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especially important for clades in which the loss of a relatively small number of species can lead to a disproportionately high loss of phenotypic diversity (e.g., Roy, Balch, & Hellberg, 2001).

In this paper, we focus on the morphological diversity of pollen grains produced by the flowering plants (including trees, woody shrubs, and herbs) in a Neotropical rainforest (Barro Colorado Island, Panama (BCI)). Pollen grains represent a discrete haploid phase in the life cycle of seed plants and serve to transfer the male gametes to the female reproductive organs. They are a critical component of plant reproduction, modulating gene flow, and providing nutrition for some animal pollination vectors. The morphological diversity of pollen grains produced by tropical rainforest plants is extremely high (see Colinvaux, De Oliveira, & Moreno, 1999; Gosling, Miller, & Livingstone, 2013 for atlases of pollen grains from Amazonia and tropical West Africa, respectively, see Bush & Weng, 2007; Vincens et al., 2007; Jaramillo & Rueda, 2020 for online databases of pollen from tropical regions, and see Roubik & Moreno, 1991; Mander, 2016 for monographic and theoretical treatments of BCI pollen morphology, respectively). Pollen grains are well represented in the fossil record and provide key data on the diversification history of tropical vegetation (van der Hammen, 1954; Livingstone, 1962; Jardiné & Magloire, 1963; Germeraad, Hopping, & Muller, 1968; Hooghiemstra, 1984; Muller et al., 1987; Bush & Colinvaux, 1990; Colinvaux, De Oliveira, Moreno, Miller, & Bush, 1996; Morley, 2000; Jaramillo, Rueda, & Mora, 2006). However, despite their importance in plant reproduction and as a record of ancient plant diversity, it is unclear whether the morphological variety of pollen grains reflects evolutionary descent or ecological function.

In a phylogenetic context, pollen morphology is highly diagnostic of certain clades. For example, grass (Poaceae) pollen grains are characterized by fine-scale channels in the exine, and this is a morphological character that separates the Poales from the Restionales (Linder & Ferguson, 1985). However, it is often the case that pollen morphology is similar or invariant within major clades (von Post, 1916; Birks & Birks, 2000; Cain, 1940; Mander & Punyasena, 2014). For example, virtually all genera within Poaceae produce pollen of similar form regardless of their phylogenetic distance from one another (Kohler & Lange, 1979; Mander, Li, Mio, Fowlkes, & Punyasena, 2013; Page, 1978; Wodehouse, 1935). Examples of other global plant families present in tropical forests in which pollen morphology is uniform include the Lauraceae and Moraceae, which produce spherical inaperturate pollen and oblate diporate pollen, respectively (Roubik & Moreno, 1991; Burn & Mayle, 2008; Mander, 2016; see Punt, Hoen, Blackmore, Nilsson, & Le Thomas, 2007 for terminology).

Work on the functional biology of pollen presents an inconsistent picture of how pollen morphology relates to plant ecology. Some authors have noted that "every detail of the exine may serve a functional purpose" (Punt, 1986, p. 97), and others who have focussed on the role of pollen morphology in pollination have suggested that there "is a strong negative correlation between the presence of exine ornament and wind dispersal of the pollen" (Chaloner, 1976, p. 3). However, processes such as the dispersal of pollen are thought to be mediated by flower morphology and plant

reproductive strategy rather than pollen morphology. For example, in rainforests the pollen of dioecious plants that have open flowers in order to allow small generalist insects to access anthers are over-represented in pollen traps compared with the occurrence of source plants in local forests (as measured by percentage basal area), whereas the pollen of hermaphroditic plants is under-represented in pollen traps (Bush, 1995; Bush & Rivera, 1998, 2001). Additionally, it has been noted that certain morphological features of pollen grains may have multiple functional roles; for example, apertures regulate water ingress and egress, and are sites where substances may be transported between pollen and stigma (Heslop-Harrison, 1976), but they may also serve as sites of pollen tube exit during germination in some (but not all) taxa (Chaloner, 2013), and guide folding pathways during the desiccation phase of pollen dispersal (Katifori, Alben, Cerda, Nelson, & Dumais, 2010). Such a broad range of functions associated with the micro-morphological features of pollen grains have led some authors to conclude that pollen grains are "not 'optimally' designed for a specific function, but merely structures that work with varying efficiency in a specific ecological and evolutionary context" (Crane, 1986, p. 184).

This body of previous work has been unable to reach consensus on the degree to which pollen morphology reflects evolutionary descent versus ecology, which prevents understanding why pollen grains are characterized by such striking diversity and hampers knowledge of the drivers of morphological diversity in plants generally. We address this issue by presenting a case study of the relationship between the pollination ecology, molecular evolution, and morphological diversity of the pollen grains of flowering plants on BCI. Previous phylogenetic work has demonstrated the feasibility of constructing community phylogenies for the plants of BCI (Kembel & Hubbell, 2006; Kress et al., 2009). We build on this body of work by reconstructing a more comprehensive phylogeny of angiosperms on BCI, combining data on the pollination ecology of these plants, and quantitative measurements of the morphological diversity of their pollen grains. Our specific aims are to: (a) compare the morphological distance between pollen morphotypes with branch-length genetic distance among BCI angiosperms; (b) examine the relationship between morphological diversity and pollination strategy on BCI; and (c) to identify the morphological traits that are characteristic of different pollination strategies on BCI.

2 | METHODS

2.1 | Phylogeny of BCI angiosperms and genetic distance measurement

We constructed a molecular phylogeny encompassing 730 species of the 759 genera and 1,263 lesser taxa of angiosperms known from BCI (Croat, 1978) (Figure S1). DNA sequence data were obtained from GenBank using the PHLAWD system (Smith & Walker, 2019). Four chloroplast regions, *rbcl*, *trnL*, *ndhF*, and *matK* were used alongside the nuclear internal transcribed spacer (ITS) region. These sequences were aligned using MAFFT version 7 (Katoh & Stanley,

2013) and concatenated into a supermatrix. The maximum-likelihood BCI angiosperm phylogeny was reconstructed in RAxML, version 8 (Stamatakis, 2014) using the general time reversible model with Gamma site heterogeneity.

There are several standard calculations of phylogenetic diversity (for a review of approaches see Vellend et al., 2011). Each of these describes the degree of phylogenetic relatedness either between individual pairs of species, or on average across an entire phylogeny. One metric that has been used previously to compare phylogenetic relatedness with morphological distance is the branch-length distance (BLD) metric (Kelly et al., 2014). BLD is calculated as the sum of the branch lengths on a molecular phylogeny scaled to units of average molecular substitutions per site. As an estimate of the molecular divergence between pairs of species, the BLD is also a measure of genetic distance that is informed by phylogeny and adjusted according to a Markovian model of sequence evolution.

To compute BLD, we pruned from the 730-taxon tree species without adequate data on pollen morphology. This resulted in a 648-taxon tree, with branch lengths measured in units of nucleotide substitutions per site. Molecular distances were then calculated by summing the lengths of the branches (in units of nucleotide substitutions) when tracing the path separating each pair of taxa in the tree. This yielded a measure of phylogenetic diversity that also accommodates differences in rates of molecular evolution. As a result, the metric captured the phylogenetic distance between pairs of taxa, weighted by the amount of evolution expected based on the patterns in molecular divergence reconstructed from the phylogeny.

2.2 | Pollination strategy of BCI angiosperms

We searched the published literature for information on the pollination strategy of BCI angiosperms (Ackerman, 2000; Bawa, 1977; Bawa, Bullock, Perry, Coville, & Grayum, 1985; Bentley, 1977; Bittrich, Amaral, & Melo, 1993; Bullock, 1994; Bush, 1995; Bush & Rivera, 1998, 2001; Colin & Eugene Jones, 1980; Collevatti, Schoereder, & Campos, 2000; Datta, Maiti, & Basak, 1982; de Miranda & Reinhard, 2006; Estes & Thorp, 1974; Huang, 2003; Le Corff, Agren, & Schemske, 1998; Martins & Batalha, 2006; Molano-Flores, 2001; Oliveira, Gibbs, & Barbosa, 2004; Pilatowski, 1982; Renner & Feil, 1993; Ribeiro et al., 1999; Sakai, Kato, & Nagamasu, 2000; Simmonds, 1945; Smith, Mori, Henderson, & Stevenson, 2004; Stacy et al., 1996). Since there were very few direct studies on the pollination strategy of individual species, we used family- and genus-level information to place 992 BCI angiosperm species into the categories of biotic/abiotic/mixed pollination (Tables S1 and S2).

2.3 | Description of pollen morphology and measurement of morphological distance

Pollen morphology data for each angiosperm taxon in our phylogeny and pollination strategy datasets were extracted from Mander

(2016), which describes angiosperm pollen grains from BCI plants. The Mander (2016) data were derived from a monograph containing morphological descriptions and images of BCI pollen grains (Roubik & Moreno, 1991) by scoring each pollen grain for the following six discrete morphological characters: apertures (60 character states), exine stratification (4 character states), shape (10 character states), dispersal unit (6 character states), primary surface ornamentation (22 character states), and secondary surface ornamentation (23 character states) (see Punt et al., 2007 for terminology).

For each pollen type, these six characters were concatenated so that the pollen morphology of each taxon was represented by a string of length 6. Each unique string constitutes a pollen morphotype. The morphological distance between taxa was measured by computing the Hamming distance between their strings using a script written in the Python programming language (following Mander, 2018, see the Supplementary Information). The Hamming distance between two strings of equal length can be defined as the number of positions at which the corresponding characters are different (Hamming, 1950). For example, the following two taxa and their strings (in brackets) are from the dataset used in this paper:

Piper aequale (Piperaceae) = (19, 4, 7, 1, 2, 23).

Prioria copaifera (Fabaceae) = (44, 3, 8, 1, 8, 19).

In the language of descriptive palynology (Punt et al., 2007), these strings contain the following morphological information. The pollen of *Piper aequale* is monocolpate (19) with a tectate exine (4) and suboblate shape (7) that is dispersed as a monad (1) and has scabrate primary surface ornamentation (2) and lacks secondary surface ornamentation (23). In contrast, the pollen of *Prioria copaifera* is tricolpate (44) with a semitectate exine (3) and subprolate shape (8) that is dispersed as a monad (1) and has verrucate primary surface ornamentation (8) and perforate secondary ornamentation (19).

The two strings that encode the morphology of these taxa, (19, 4, 7, 1, 2, 23) and (44, 3, 8, 1, 8, 19) differ at five positions, and so the Hamming distance between them is 5. In this paper, the Hamming distance is reported as a proportion, and this is equivalent to 1–SMC, where SMC is the simple matching coefficient that has been used in previous studies of morphological diversity (Foote, 1994; Lupia, 1999). In this paper, we use the term morphological diversity, which is a synonym of the term morphological disparity that has been used by other authors (e.g., Foote, 1994; Lupia, 1999).

2.4 | Statistical tests

Statistical significance tests were performed in R (R DCT, 2013). We tested the association between morphological and genetic distance using Spearman's ρ , which is non-parametric and computed using `cor.test(x, y, method="spearman")`. We tested for differences in the morphological diversity of each pollination group—abiotic, mixed, biotic—using pairwise Wilcoxon rank sum tests, which are non-parametric and computed using `wilcox.test(x, y)` with continuity

correction and without p -value adjustment. We visualized the distribution of taxa in morphological space using a linear discriminant analysis, which was performed in the R package MASS using `lda(x, data)`. We standardized sample sizes across each pollination group using bootstrap analyses (random resampling with replacement) of mean pairwise morphological diversity and the proportion of unique pollen morphotypes, which were performed using a script written in the Python programming language (see the Supplementary Information).

3 | RESULTS

Morphological distance was measured by the Hamming distance between pollen morphotypes (defined by character strings of length 6, see Methods) and is reported as a proportion. When the Hamming distance between two strings of length 6 is computed, the following 7 possible values are possible: 0 (strings identical), 0.17 (one position differs), 0.33 (two positions differ), 0.50 (three positions differ), 0.67 (four positions differ), 0.83 (five positions differ), and 1 (all positions differ). The morphological distance between the taxa in our phylogeny varies from 0 to 0.83 (Figure 1); however, the majority of taxa are separated by a morphological distance of 0.33–0.67, and there are relatively few taxa that are either morphologically identical or are separated by a morphological distance of 0.17 or 0.83 (Figure 1). There is considerable variation in the genetic distance of plants that have morphological distance values of 0.33 and 0.50, whereas plants that have morphological distance values of 0 and 0.83 have less variation in their genetic distances (Figure 1). As a group, taxa that are separated by a morphological distance of 0.83 are all further apart genetically than taxa that are identical in terms of their pollen morphology (Figure 1). The association between morphological and genetic distance is statistically significant (Figure 1, Spearman's $\rho = 0.097$, $p = .014$).

In our dataset, there are 14 families and 125 species in the abiotic pollination category, 6 families and 72 species in the mixed pollination category, and 104 families and 795 species in the biotic pollination category (Table 1). As a group, plants that are biotically pollinated on BCI have higher morphological diversity than plants that have a mixed pollination strategy or are abiotically pollinated (Figure 2), and the differences in morphological diversity among these three groups are statistically significant (Wilcoxon rank sum tests, abiotic–mixed: $W = 8,139,600$, $p < .001$; abiotic–biotic: $W = 544,190,000$, $p < .001$; mixed–biotic: $W = 254,000,000$, $p < .001$). Mean pairwise morphological diversity is highest in biotically pollinated plants and lowest in abiotically pollinated plants (Figure 2).

There are many more biotically pollinated taxa in BCI compared to wind-pollinated taxa (Table 1). However, these two groups have a very similar number of families as a proportion of the number of species, with biotically pollinated plants having 104 families and 795 species (0.13) and abiotically pollinated plants having 14 families and 125 species (0.11) (Table 1). To account for the considerable differences in the numbers of taxa in each pollination group, we performed

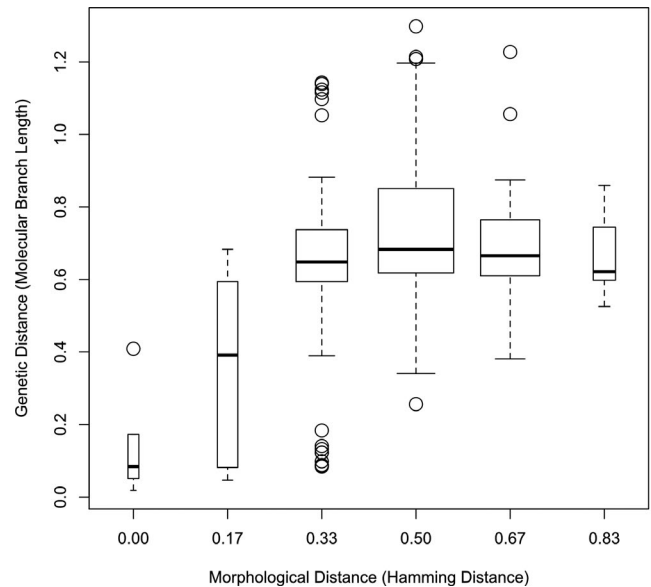


FIGURE 1 Comparison of the morphological and genetic distance between plants on Barro Colorado Island. Genetic distance measured by molecular branch lengths (substitutions per site). Morphological distance measured by the Hamming distance between pollen morphotypes. Width of each boxplot proportional to the square root of the number of datapoints each group

a bootstrap analysis with 1,000 replicates, each containing 50 randomly selected taxa per pollination group in order to standardize sample size. The abiotic pollination group is not only represented by one or two classic wind-pollinated families such as the grasses, and the result of this bootstrap analysis also indicates that morphological diversity is highest in biotically pollinated plants and lowest in abiotically pollinated plants (Figure 3) (Wilcoxon rank sum tests, abiotic–mixed: $W = 26,162$, $p < .001$; abiotic–biotic: $W = 0$, $p < .001$; mixed–biotic: $W = 0$, $p < .001$).

A linear discriminant analysis indicates that these three groups overlap in morphological space (Figure 4). The three groups do not form discrete clusters, and there are representatives of each pollination strategy distributed throughout morphological space (Figure 4, see also Figure S2 and Figure S3). Despite this, the abiotic and biotic pollination groups both contain a high number of unique pollen morphotypes. In our dataset of BCI angiosperms, there are 125 abiotically pollinated plants and these produce 47 pollen morphotypes, 36 of which (77%) are unique to this pollination strategy and are not produced by plants that employ a mixed or biotic pollination strategy (Table 1). Similarly, there are 795 biotically pollinated BCI plants in our dataset and these produce 406 pollen morphotypes, 386 of which (95%) are only found in this pollination group (Table 1). Since the numbers of taxa in each pollination group vary considerably, we undertook a resampling analysis in which 25 taxa were randomly selected from each pollination group and the proportion of unique pollen morphotypes in each group was calculated. We standardized the sample size to 25 because there are just 30 pollen morphotypes in the mixed pollination group. This analysis was repeated with 1,000 bootstrap replicates, and the results indicate that each pollination

TABLE 1 Summary of the number of taxa per pollination strategy studied here, together with the number and proportion of pollen morphotypes that are unique to each pollination strategy on Barro Colorado Island

Pollination strategy	Number of families	Number of species	Total number of pollen morphotypes produced	Number of unique pollen morphotypes	Proportion of unique pollen morphotypes	Min–Max proportion of unique pollen morphotypes ($n = 25, 1,000$ bootstrap replicates)
Abiotic	14	125	47	36	0.77	0.8–1.0
Mixed	6	72	30	16	0.53	0.76–1.0
Biotic	104	795	406	386	0.95	0.84–1.0

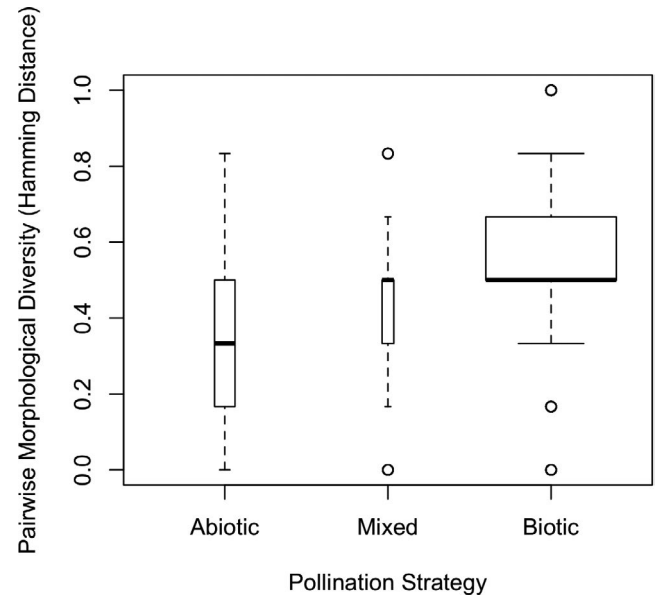


FIGURE 2 The morphological diversity of plants with abiotic, mixed, and biotic pollination strategies as measured by pollen grains. Morphological diversity measured as the Hamming distance between all pairs of taxa in each pollination group. The abiotic group contains 125 [$n(n-1)/2 = 7,750$ unordered pairs], the mixed group contains 72 taxa (2,556 unordered pairs), and the biotic group contains 795 taxa (315,615 unordered pairs). Width of each boxplot proportional to the square root of the number of unordered pairs in each group

group contains a considerable proportion of unique pollen morphotypes (Figure 5).

4 | DISCUSSION

4.1 | Morphological and genetic distance among BCI angiosperms

Many plant clades produce pollen grains that are essentially the same in terms of their gross morphological characteristics. For example, pollen grains that have tricolporate apertures and reticulate surface ornamentation (see Punt et al., 2007 for terminology) are produced by at least 28 families on BCI, including certain taxa from families separated by considerable evolutionary distance such as Euphorbiaceae (Fabales), Malvaceae (Malvales), and Bignoniaceae (Lamiales) (Jaramillo & Rueda, 2020; Mander, 2016; Roubik & Moreno, 1991). The pollen grains of these taxa also frequently possess a tectum and are dispersed as single grains rather than united in tetrads, and in such cases, these taxa share four of the six discrete characters used to describe pollen morphology in this analysis.

Such morphological similarity among relatively distant clades (Figure 1) may be explained partly by convergent evolution, as has been shown for pollen shape in Myrtaceae and Onagraceae (Kriebel, Khabbazian, & Sytsma, 2017), but may also be due partly to the coarseness of the discrete characters employed here. For example,

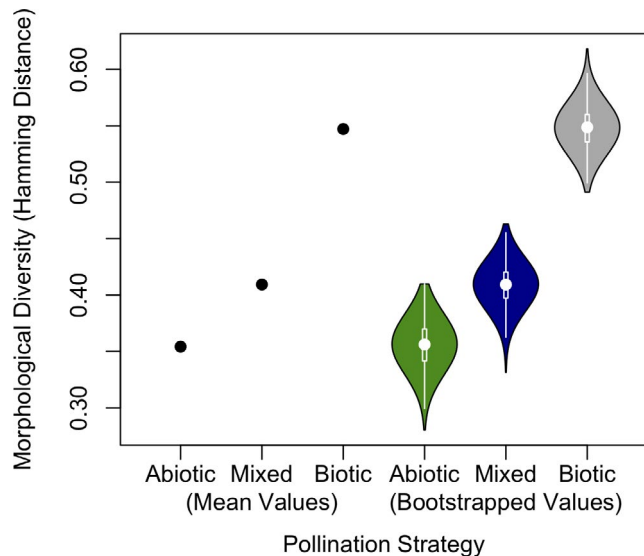


FIGURE 3 The morphological diversity of pollen grains produced by plants with abiotic, mixed, and biotic pollination strategies reported as pairwise Hamming distance. Plot shows the mean morphological diversity of each group as solid black circles (abiotic group $n = 125$, mixed group $n = 72$, biotic group $n = 795$), and the bootstrapped mean morphological diversity of each group as colored violin plots (1,000 bootstrap replicates with 50 randomly selected specimens per pollination group). The height of each violin displays the range of bootstrapped calculations of morphological diversity in each pollination group, and the width of each violin shows the frequency of bootstrapped calculations of morphological diversity within each pollination group. Boxplots within each violin show the median, and interquartile range of bootstrapped calculations of morphological diversity within each pollination group

pollen grains were assigned one of 22 different classes of primary surface ornamentation but subtle variations within each of these classes were not captured. In the case of Asteraceae (daisy) pollen, for example, the pollen surface is covered by pronounced and distinctive conical spines, but in some taxa the spines are distributed densely and in others the spines are distributed sparsely. The characters used here do not capture such differences in surface ornamentation density, and similar variations in the nature of the other characters are also not captured. The use of more characters, either describing features not considered here or accounting for variations in the nature of features such as surface ornamentation, would increase the number of possible Hamming distance values between any two taxa, which may in turn smooth the distribution shown in Figure 1 and more finely differentiate taxa.

The relationship between morphological and genetic distance found in our analyses of BCI angiosperms (Figure 1) has the same non-linear form found in simulations and meta-analyses of biological feature matrices (Kelly et al., 2014). In particular, our results support the idea that morphological distance does not always increase monotonically with genetic distance; and while close relatives share more features than distant relatives (the distribution of genetic distance measurements for Hamming distance values of 0.00 and 0.83

do not overlap, Figure 1), "beyond a certain threshold increasingly more distant relatives are not more divergent in phenotype" (Kelly et al., 2014, p. 600; see also Letten & Cornwell, 2015). For our data, this threshold is at a genetic distance of ~ 0.7 (Figure 1), which means

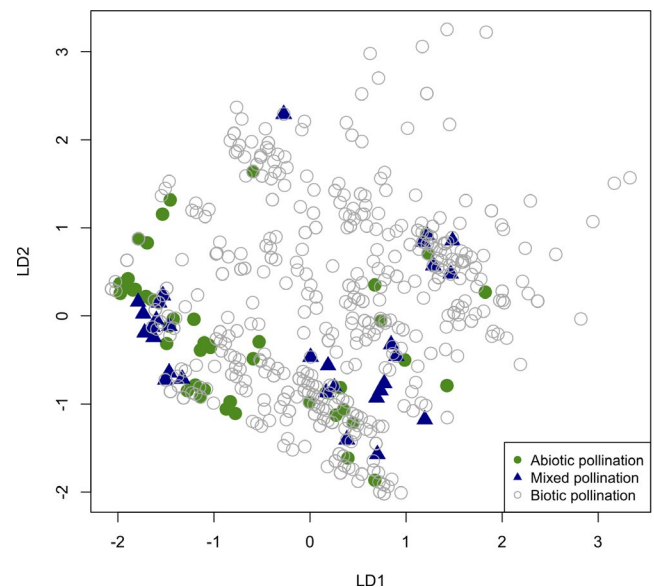


FIGURE 4 Scatterplot summarizing a linear discriminant analysis of the pollen morphology of the 992 plant species for which pollination strategy data were collected. Proportion of trace: LD1 0.9199, LD2 0.0801. See Figure S2 and Figure S3 for stacked histograms of the first and second discriminant functions, respectively

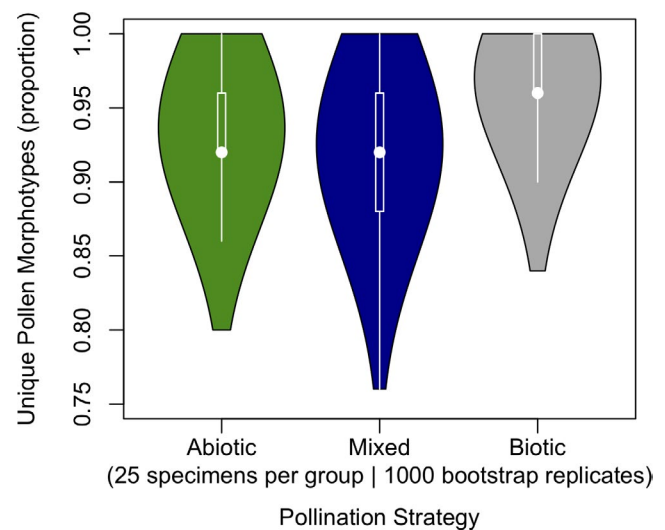


FIGURE 5 The proportion of unique pollen morphotypes within each pollination group shown as colored violin plots (1,000 bootstrap replicates with 25 randomly selected pollen morphotypes per group). The height of each violin displays the range of values and the width of each violin shows the frequency of values. Boxplots within each violin show the median, and interquartile range of bootstrapped proportions within each pollination group

that evolutionary relationships are generally a poor predictor of phenotypic differences among the pollen grains of BCI angiosperms and that the phylogenetic signal of morphology is weak in this study system (cf. Losos, 2008; Mouquet et al., 2012). It is possible that the relatively small number of morphological traits analyzed here could obscure genetic differences that evolved elsewhere in the phylogeny, and so the weak phylogenetic signal of pollen morphology can be tested with other plant organs such as leaves and flowers, as well as the branching architecture of these plants and integrative measurements of morphology that incorporate all of these features.

From the perspective of conservation biology, our results indicate that strategies founded on the idea of preserving the maximum amount of evolutionary history possible (Forest et al., 2007; Isaac, Turvey, Collen, Waterman, & Baillie, 2007) do not necessarily preserve the most diverse set of phenotypes (Kelly et al., 2014). This decoupling of phylogenetic and morphological diversity means that for clades with high morphological diversity compared to their species richness, the extinction of a relatively small number of species could lead to a disproportionately high loss of phenotypic diversity (e.g., Roy et al., 2001). Given that the conservation of phenotypic diversity is known to be important for maximizing contemporary evolutionary novelty and future evolutionary trajectories (Kelly et al., 2014), our results support calls for independent measures of morphological variety in assessments of biodiversity (Roy et al., 2001; Roy & Foote, 1997).

4.2 | Morphology and pollination ecology on BCI

Flowers are composed of distinct components, including the perianth, androecium, and gynoecium, and variation in the nature of these components such as changes in merism, hypanthium type, and floral symmetry, contributes to the overall morphological diversity of a floral display in a community of plants. This morphological variation has a demonstrable impact on the interactions between plants, pollinators, and ecological context. For example, the flowers of *Heliconia caribea* (Heliconiaceae) are relatively short and are primarily pollinated by short-billed male purple-throated hummingbirds, whereas the flowers of *H. bihai* are relatively long and are primarily pollinated by long-billed female purple-throated hummingbirds (Temeles et al., 2016). Additionally, pollination is facilitated among different co-flowering plant species that have morphologically distinct flowers (Ghazoul, 2006).

In our dataset, many more taxa employ a biotic pollination strategy ($n = 795$) than an abiotic strategy ($n = 125$) (Table 1) and consequently comparing the numbers of morphotypes produced by each group is difficult. Our classifications of biotic and abiotic pollination strategies are based on a limited number of pollination studies and because wind pollination may not be a viable strategy for plants that occur in low population densities, or in the relatively windless forest understory, it is possible that we have overestimated abiotic pollination. Even with those caveats, however, the abiotically pollinated plants in our dataset produce just 47 pollen morphotypes (compared

to 406 produced by biotically pollinated plants), but 36 (77%) of these morphotypes are unique to this pollination strategy (Table 1, see also Figure 5). This indicates that the morphotypes produced by plants in the abiotic group are not simply a limited subset of the morphotypes produced by the more abundant biotically pollinated plants. Indeed, 95% of the pollen morphotypes produced by biotically pollinated plants are unique to this pollination strategy, and there are also a high number of individual morphological traits that are unique to the plants in this group. For example, of the 40 aperture types in our data, 24 (60%) of them are only produced by biotically pollinated plants, and 4 out of 5 pollen dispersal units (80%) are only produced by plants with this pollination ecology (Table S3). Despite the uniqueness of morphotypes in each pollination group, the pollen grains of biotically pollinated and abiotically pollinated plants do overlap in morphological space (Figure 4). One reason for this apparent paradox is that a difference in just a single character is sufficient for a morphotype to be unique, but a difference in a single character is not sufficient for a morphotype to be widely separated from the rest of a population in morphological space. Additionally, the distribution of BCI pollen grains in morphological space represents a snapshot at the present day. It is possible that the three pollination groups we have studied here have always overlapped in morphological space. However, it may be that recent diversification of pollen morphologies has homogenized a previous distribution that was once more segregated (Erwin, 2007; Gould, 1989) or, given that pollen morphology is frequently conserved, it could also be that different clades with similar pollen morphologies have shifted their pollination strategy during recent radiation, which would also produce an overlap between their mode of pollination and their pollen morphology. A phylomorphospace analysis (e.g., Santos, Perrard, & Brady, 2019) may go some way to exploring this avenue further.

Taken together, the partitioning of pollen morphotypes and individual morphological traits among pollination groups (Table 1, Table S3), as well as the high morphological diversity among the pollen of biotically pollinated plants on BCI (Figures 2 and 3), indicate that the micro-morphological landscapes of biotically and abiotically pollinated plants are considerably different in this flora. Viewed in this way, pollen grains contribute to the overall phenotypic diversity that is presented by plants to pollinators, and this may represent a link between pollination ecology and pollen morphology on BCI. Such partitioning of pollen morphotypes and individual micro-morphological traits between biotically pollinated and abiotically pollinated plants has been widely reported in previous work on pollen morphology in the context of pollination ecology. For example, "wind-pollinated [Asteraceae] genera *Ambrosia* and *Artemisia* have generally smooth dry pollen in contrast to the more widespread insect-pollinated genera of the family which have ornamented, often sticky pollen grains" (Ferguson & Skvarla, 1982, p. 189). Similarly, while the grasses are thought of as a classic example of a wind-pollinated plant group (e.g., Wodehouse, 1935), two species of phorid flies (Phoridae) and gall midges (Cecidomyiidae) have been observed spending their life cycles on the Bambusoid grass *Pariana* in tropical rainforests, and these insects may be responsible for the pollination of this genus in rainforests (Soderstrom & Calderon, 1971). The

pollen of grasses is generally characterized by a subtle and reduced surface ornamentation (Mander et al., 2013), but the pollen of the insect-pollinated *Pariana* is unique in having considerably coarser surface ornamentation than other grasses (Mander & Punyasena, 2015). Similar associations have been noted between modes of pollination and pollen size. For instance, many fossil angiosperm pollen grains from early in the evolution of this plant group in the Early Cretaceous are very small, but by the Middle and Late Cretaceous larger pollen grains appear in the fossil record, and it has been suggested that "this increase in size was primarily caused by increased adaptation to different systems of animal pollination" (Muller, 1979, p. 610).

However, for each example of a correlation between pollen morphology and mode of pollination, there is a counter example showing no relationship between the two. For instance, as the case of *Ambrosia* and *Artemisia* shows, wind-pollinated plants tend to have smooth and dry pollen while insect-pollinated plants have coarsely ornamented and oily pollen, but in some insect-pollinated plants, such as *Viola* the pollen is uniformly smooth and dry (Beattie, 1971). Additionally, the link between pollen morphology and pollination ecology is not easy to reconcile with current knowledge of pollen functional biology. This is, at least in part, because the individual morphological traits of pollen grains, such as apertures, exine stratification, and surface ornamentation, may each serve multiple functions that are not all related to pollination. For example, it has been suggested on the basis of experimental work that exine sculpture may play a role in the involvement of static electrical charge in the transfer of pollen from the pollination vector to the stigma (Chaloner, 1986), but it has also been demonstrated that prominent sculptural elements on the pollen surface such as club-shaped clavae prevent mirror buckling when pollen is desiccated (Katifori et al., 2010).

In contrast to the debate surrounding the relationship between individual morphological traits of pollen grains and pollination ecology, the observation of high morphological diversity among biotically pollinated plants compared to abiotically pollinated plants (Figures 2 and 3) has not been previously reported or quantified. Our results are not able to distinguish whether this is a consequence of selection for high morphological diversity among the pollen grains of biotically pollinated plants, or because of constraints on the pollen morphology of wind-pollinated plants that may be mechanical or related to dispersal in air. However, by analogy with the role of morphological diversity among flowers (e.g., Ghazoul, 2006), and rather than attributing a particular set of pollen morphotypes or individual morphological traits to a particular pollination ecology, it could be hypothesized that pollen grains may contribute to the overall phenotypic diversity that is presented by plants to pollinators.

The viability of this hypothesis would require a demonstration that animal pollination vectors use morphology as a cue during pollen foraging, but at present there is no published information that explicitly examines the degree to which different animal pollinators are able to perceive micro-morphological differences between pollen grains. It seems unlikely that a hummingbird, for example, would be able to perceive the difference between a pollen grain that has

sculptural elements shaped as conical spines (echinae) rather than cylindrical rods (baculae), but recent work on bees has concluded that "considering their diverse sensory capabilities, from a bees' perspective pollen represents a multimodal stimulus, at once providing foragers with gustatory, olfactory, visual and mechanosensory cues, all of which could be used to guide their foraging choices" (Nicholls & Hempel de Ibarra, 2017, p. 81). For example, it has been shown experimentally that bumble bees (*Bombus terrestris*) performed significantly less pollen foraging on plants which have large, echinate pollen grains (three Malvaceae species and *Knautia arvensis* (Dipsacaceae)) (Konzmann et al., 2019), but studies of pollen foraging on *Cucurbita* (Cucurbitaceae) by the generalist bumble bee *Bombus impatiens* have shown it is likely that combinations of morphological (large size and pronounced echinae in this example), nutritional and chemical pollen traits could allow plants to selectively attract and deter particular suites of pollinators (Brochu et al., 2020). It seems unlikely that pollinators could perceive subtle differences in pollen morphology, especially those on a micrometric scale, but further experimental work could test whether the micro-morphological landscape provided by pollen grains is functionally significant for bees and other insects during pollination. Such work could help to establish the ecological significance of pollen morphology in our study system (cf. Losos & Miles, 1994; Miles & Ricklefs, 1984; Ricklefs, 2012).

In addition to its potential role in pollination success, it is possible that certain pollen morphologies could be linked to differences in pollen production by plants employing different pollination strategies and life strategies. Although speculative, future work could consider, for example, whether certain pollen morphologies are characteristic of plants that are commonly over-represented in pollen trapping studies on BCI such as the treefall gap colonists *Cecropia* and *Trema* together with *Alchornea* and certain members of the Solanaceae and Malpighiaceae (Bush, 1995; Bush & Rivera, 2001; Haselhorst, Moreno, & Punyasena, 2013). Finally, certain morphological features may simply arise by self-assembly processes that reflect convergence on a minimum free-energy state (Hemsley, Collinson, Kovach, Vincent, & Williams, 1994); recent biophysical modeling has shown that surface ornamentation patterns may form through the phase separation of a polysaccharide layer that is mechanically linked to a cell membrane during pollen wall development (Radja, Horsley, Lavrentovich, & Sweny, 2019). Currently, there is insufficient data on the distribution of these biophysical processes across angiosperm clades to enable us to link them to the pollination biology or phylogenetic history of BCI angiosperms, but establishing this link represents a possible avenue of future work.

5 | CONCLUDING REMARKS

Our study indicates that the morphological diversity of the pollen grains of flowering plants in a Neotropical rainforests can be related to phylogeny and also perhaps to pollination ecology. The micro-morphological features of pollen grains show constraints from evolutionary descent, but the relationship between morphological

and branch-length genetic distance is non-linear (Figure 1). Close relatives share more morphological features than distant relatives but above a genetic distance of ~ 0.7 increasingly distant relatives are not more divergent in phenotype (Figure 1); this highlights that morphological distance does not always increase monotonically with genetic distance (Kelly et al., 2014; Letten & Cornwell, 2015). Pollen morphology may also be related to pollination ecology on BCI. The pollen grains of biotically pollinated and abiotically pollinated plants overlap in morphological space (Figure 4), but there is evidence that pollen morphotypes and individual morphological traits are partitioned among these pollination ecologies (Table 1, Table S3). The pollen of biotically pollinated plants on BCI is more morphologically diverse than pollen of abiotically or mixed pollinated plants, but the relatively low numbers of taxa in the abiotic pollination group make comparison difficult (Figures 2 and 3). Moreover, it is important to note that morphological traits of pollen grains may serve functions unrelated to pollination. Our results are not able to distinguish whether differences in morphological diversity are because of selection for high morphological diversity among pollen grains of biotically pollinated plants, or constraints on the pollen morphology of wind-pollinated plants. However, by analogy with the role of morphological diversity among flowers in pollination (e.g., Ghazoul, 2006), we hypothesize that pollen grains may contribute to the overall phenotypic diversity that is presented by plants to pollinators. This hypothesis provides a link between pollination ecology and pollen morphology and could be tested using foraging experiments such as those of Konzmann, Koethe, and Lunau (2019) and Brochu et al. (2020).

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CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

AUTHOR CONTRIBUTION

L.M. and C.J. designed the study. C.P-F. and C.W.D. undertook phylogenetic analyses and measurements of genetic distance. S.W.P. and C.J. gathered data on pollination strategy. L.M. measured morphological distances among pollen grains. L.M. wrote the paper with input from all authors.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.59zw3r25b> (Mander, Parins-Fukuchi, Dick, Punyasena, & Jaramillo, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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